

# Antimutagenic and Mutagenic Potentials of Chinese Radish

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The edible part of fresh Chinese radish was chopped into small pieces, lyophilized, and then extracted sequentially with hexane, chloroform, and methanol. The solvent in each fraction was removed by evaporation under reduced pressure at 50–55°C, and the residue was dissolved in dimethylsulfoxide just before being tested for antimutagenicity as well as mutagenicity using the Salmonella/mammalian microsome mutagenicity test. We found that none of the three fractions exhibited any mutagenicity toward *S. typhimurium* strains TA98 and TA100 when tested either in the presence or absence of S-9 mix. Interestingly, however, hexane and chloroform extracts could strongly inhibit the mutagenicities of both direct mutagens (e.g., 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide and sodium azide) and indirect mutagens (e.g., aflatoxin B<sub>1</sub>). In contrast, however, these two fractions did not inhibit the mutagenicity of benzo[a]pyrene, which is also an indirect mutagen. Both hexane and chloroform extracts could also markedly inhibit the activities of rat liver aniline hydroxylase and aminopyrine demethylase. The methanol fraction could inhibit neither the mutagenicities of direct or indirect mutagens tested nor the activities of those two rat liver enzymes. Results of the present study demonstrate that Chinese radish may not contain any mutagenic compound but does contain some nonpolar compounds with antimutagenic activity toward both direct and indirect mutagens. In addition, the antimutagenic activity toward aflatoxin B<sub>1</sub> may be partly due to the inhibition of enzymes necessary for activation of this mutagen.

## Introduction

It has been recommended for a number of years that we should increase our consumption of cruciferous vegetables because there is some evidence from epidemiological studies that increased intake of these vegetables, which include cabbage, Brussels sprouts, broccoli, and cauliflower, is associated with reduced risk of cancer of the colon, rectum, and bladder (1,2). Results of another prospective cohort study also demonstrated that subjects who had a higher than mean intake of broccoli and Brussels sprouts were at decreased overall risk of death from cancer (3). Furthermore, cabbage and cauliflower have been shown to inhibit the preneoplastic response and hepatic tumorigenesis induced by aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) (4–6) and inhibit mammary gland tumorigenesis induced by dimethylbenzanthracene (DMBA) (7) in rats when added to the diet. Some indoles naturally occurring in cruciferous vegetables have also been shown to inhibit the DMBA-induced mammary gland tumor formation in rats and benzo[a]-

pyrene (BaP)-induced neoplasia of the forestomach in mice, presumably by inducing the mixed-function oxidase system (8). In addition, R-601, which is also found in these vegetables, has been shown to increase the hepatic glutathione-S-transferase activity and biliary excretion of AFB<sub>1</sub>-glutathione conjugates and decrease the binding of AFB<sub>1</sub> to hepatic DNA (9). These compounds may play roles in the anticarcinogenic effect of cruciferous vegetables. Recently, some vegetables in this Cruciferae family such as cabbage, collards, broccoli, and turnip greens have also been shown to contain antimutagens (10). Thus, the anticarcinogenic effect of these vegetables may well be due to the presence of many compounds with different properties.

Chinese radish, *Raphanus sativus* Linn., is also a member of Cruciferae family. It is commonly consumed in many Asian countries including Thailand. However, there was no information regarding either the anticarcinogenic or antimutagenic effects available for this vegetable. It is of much interest therefore to study its antimutagenic and anticarcinogenic potentials as well as the possible mechanism of the antimutagenic effect. This communication reports the antimutagenic effect of the hexane, chloroform, and methanol extracts of Chinese radish as well as the effect on the rat liver cytochrome P450-linked enzymes. Because many plants and vegetables have been found to contain mutagens (11), we also tested the mutagenic potential of this vegetable.

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## Materials and Methods

### Chemicals

Aflatoxin B<sub>1</sub> was obtained from Makor Chemicals Ltd. (Jerusalem, Israel), BaP, sodium azide (NaN<sub>3</sub>), and glucose-6-phosphate from Sigma Chemical Co. (St. Louis, MO), and 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide (AF-2) was a generous gift from M. Nagao (National Cancer Center Research Institute, Japan).  $\beta$ -NADP was purchased from Oriental Yeast, Co., Ltd. (Osaka, Japan), and polychlorinated biphenyl (PCB-54) from TCI Co. (Tokyo, Japan). Dimethyl sulfoxide (DMSO), spectrophotometric grade, was from Merck (Darmstadt, FRG) and Oxoid nutrient broth No. 2 was obtained from Oxoid Ltd. (Hants, UK). All other chemicals were mostly of analytical grade.

### Bacterial Tester Strains

*Salmonella typhimurium* strains TA98 and TA100 were kindly provided by T. Matsushima of the Japan Bioassay Laboratory. The strains were cultured in Oxoid nutrient broth no. 2 for 12 hr before use.

### Preparation of Vegetable Extracts

Chinese radish was purchased from the local markets in Bangkok. The edible part was chopped into small pieces and then lyophilized. Dry material was blended into powder and extracted sequentially with hexane, chloroform, and then methanol by macerating and shaking. After filtration, the solvent in each fraction was evaporated under reduced pressure at 50–55°C by using a rotary evaporator. The residue was dissolved in DMSO and then filtered through a 0.45- $\mu$ m sterile membrane filter disc before subjecting it to mutagenicity and antimutagenicity testing.

### Mutagenesis Assay

The *Salmonella*/mammalian microsome mutagenicity test described by Ames and his co-workers (12,13) was used in this study with some modifications. Briefly, 10–100  $\mu$ L of the extracts, 0.5 mL of S-9 mix, or 0.5 mL of 0.1 M sodium phosphate buffer, pH 7.4 (when the test was performed in the absence of S-9 mix), 0.1 mL of an overnight (12 hr) culture of bacterial tester strain, and 2 mL of molten top agar containing 50  $\mu$ M histidine and biotin were delivered, in the order given, to sterile test tubes. The contents were mixed and poured onto minimal glucose agar plates. His<sup>+</sup> revertant colonies were scored after incubation in the dark at 37°C for 42–48 hr.

The S-9 fraction used in this study was prepared from livers of male Wistar rats that had been injected IP with PCB as previously described (13). S-9 mix contained 100–200  $\mu$ L of S-9 fraction/mL and the NADPH-generating system as used by Maron and Ames (13).

### Antimutagenicity Test

The Ames' *Salmonella*/mutagenicity test as described above was also used for testing the antimutagenic activity

of the Chinese radish extracts, but only strain TA100 was used. Only 50  $\mu$ L of standard mutagen was added to the test tubes just before top agar. In this study, 0.025  $\mu$ g AF-2 and 1  $\mu$ g NaN<sub>3</sub> were used as standard mutagens when tested in the absence of S-9 mix, and 0.03  $\mu$ g of AFB<sub>1</sub> and 5  $\mu$ g of BaP were used when tested in the presence of S-9 mix.

The number of his<sup>+</sup> revertants (after subtracting the spontaneous reversions) induced by AF-2, NaN<sub>3</sub>, AFB<sub>1</sub> and BaP tested without any extract were 2178  $\pm$  302, 1347  $\pm$  106, 1291  $\pm$  100, and 1219  $\pm$  133, respectively, and were given as 100%. The percentage of remaining his<sup>+</sup> revertants in the presence of the vegetable extracts was thereafter calculated accordingly.

### Assay of the Inhibition of Rat Liver Aniline Hydroxylase and Aminopyrine Demethylase

PCB-induced rat liver S-9 fraction was used as a source of aniline hydroxylase and aminopyrine demethylase, and the activities of these enzymes were determined by the method described by Carpenter et al. (14). Inhibitory effect of vegetable extracts was examined by adding different amounts of each extract into the reaction mixture, and the percent remaining activity was calculated by comparison to the control reaction mixture containing DMSO as the solvent.

## Results

Only the edible part of Chinese radish was lyophilized and then extracted sequentially with hexane, chloroform, and methanol. The amount of the extracts obtained were approximately 0.23, 0.36, and 29.1% of the dry weight. Thus, the extractable constituents in Chinese radish were mainly polar compounds, and the nonpolar compounds that were extractable by hexane and chloroform constituted only a minor part.

### Mutagenic Potential of Chinese Radish Extracts

All three Chinese radish extracts were tested for their mutagenic potentials toward *S. typhimurium* TA98 and TA100 both in the presence and absence of S-9 mix. Results in Table 1 reveal that these extracts were not mutagenic to either tester strain whether tested in the presence or absence of PCB-induced S-9 mix. For hexane and chloroform extracts, at the dose of 2 mg/plate, the fractions exhibited some toxicity, especially to strain TA98, both in the presence and absence of S-9 mix. However, these results indicated that Chinese radish may not contain any compound that is mutagenic both TA98 and TA100.

### Antimutagenic Activity of Chinese Radish Extracts

The test for antimutagenic activity of Chinese radish extracts was performed both in the absence of S-9 mix, in

Table 1. Mutagenic activity of Chinese radish extracts on *Salmonella typhimurium* TA98 and TA100 in the presence and absence of S-9 mix.

Extract	Amount, mg	Number of His <sup>+</sup> revertants/plate			
		TA98		TA100	
		- S-9 mix	+ S-9 mix	- S-9 mix	+ S-9 mix
Hexane	0.25	33	59	163	185
	0.5	30	50	142	151
	1.0	34	37	132	147
	2.0	14 (pk)	25 (pk)	140 (pk)	166
Chloroform	0.25	40	54	179	228
	0.5	38	53	147	180
	1.0	25	45	160	188
	2.0	20 (pk)	30 (pk)	150	155
Methanol	2.0	48	51	185	209
	5.0	39	59	203	250
	10.0	33	64	187	256
	20.0	43	74	215	236
Solvent control DMSO	100 $\mu$ l	38	53	187	195
Positive controls					
AF-2	0.025 $\mu$ g	ND	ND	2112	ND
	0.25 $\mu$ g	835	ND	ND	ND
AFB <sub>1</sub>	0.03 $\mu$ g	ND	1027	ND	1463

Results are means of two separate experiments. S-9 mix contained 200  $\mu$ L/mL of PCB-induced rat liver S-9 fraction.

Abbreviations: pk, partial killing effect; DMSO, dimethyl sulfoxide; AF-2, 2-(2-furyl)-3-(5-nitro-2-furyl)-acrylamide; AFB<sub>1</sub>, aflatoxin B<sub>1</sub>.

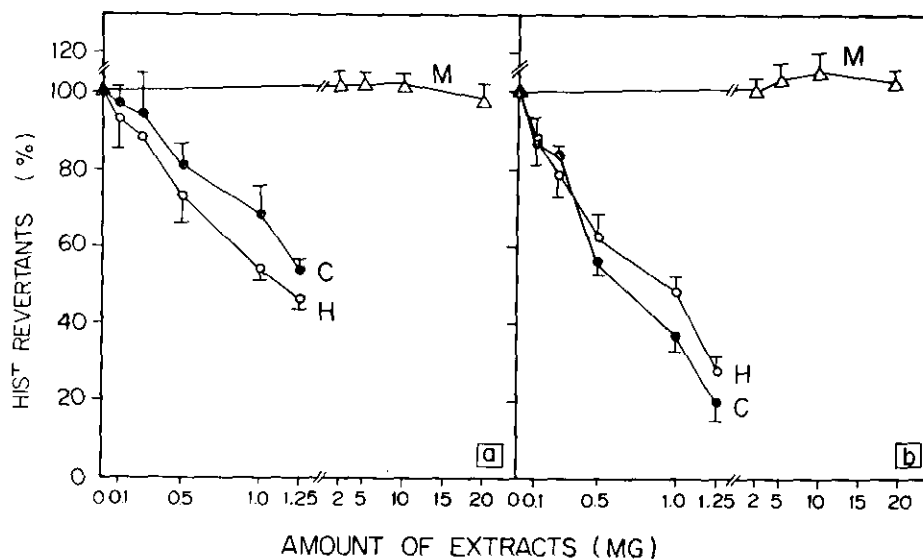


FIGURE 1. Effects of Chinese radish extracts on the mutagenicities of 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2) (a) and sodium azide (NaN<sub>3</sub>) (b) toward *S. typhimurium* TA100 in the absence of S-9 mix. The His<sup>+</sup> revertants in the presence of hexane (○), chloroform (●) and methanol (△) extracts are shown as the percentage of the His<sup>+</sup> revertants assayed without any extract. The numbers of His<sup>+</sup> revertants induced by AF-2 (0.025  $\mu$ g) and NaN<sub>3</sub> (1  $\mu$ g) in the absence of the extract were  $2,178 \pm 302$  and  $1,347 \pm 106$ , respectively. Results are means  $\pm$  SD of three separate experiments.

which AF-2 and NaN<sub>3</sub> were used as standard direct mutagens and in the presence of S-9 mix, in which AFB<sub>1</sub> and BaP were used as standard indirect mutagens. Figure 1 shows the effect of hexane, chloroform, and methanol extracts on the mutagenicities of AF-2 and NaN<sub>3</sub> toward *S. typhimurium* TA100 in the absence of S-9 mix. Hexane and chloroform extracts strongly inhibited the muta-

genicities of both AF-2 and NaN<sub>3</sub>. The amounts of hexane extract capable of inhibiting 50% of the activity of these AF-2 and NaN<sub>3</sub> were approximately 1.125 mg and 0.9 mg/plate and those of chloroform extract were approximately 1.35 mg and 0.65 mg/plate, respectively. However, at all the doses tested in this experiment, there was no toxicity to the tester strain (data not shown). On the other hand,

however, methanol extract did not show any inhibitory effect on the mutagenicity of either AF-2 or  $\text{NaN}_3$ , although it was tested up to 20 mg/plate.

Results in Figure 2 demonstrate that both hexane and chloroform extracts could also strongly inhibit the mutagenicity of  $\text{AFB}_1$  toward *S. typhimurium* TA100 when tested in the presence of S-9 mix. The amounts of these two extracts capable of inhibiting 50% of mutagenicity of  $\text{AFB}_1$  were quite similar and approximately 0.35–0.4 mg/plate. On the other hand, the methanol extract markedly increased the mutagenicity of  $\text{AFB}_1$ . This result suggested that methanol extract may contain either a co-mutagen or compound capable of increasing the metabolic activation of  $\text{AFB}_1$  to the ultimate mutagen. For BaP mutagenicity, however, all three Chinese radish extracts did not exhibit any antimutagenic effect.

### Inhibitory Effect of Chinese Radish Extracts on the Activities of Rat Liver Aniline Hydroxylase and Aminopyrine Demethylase

Figure 3 shows the effect of three Chinese radish extracts on the activities of two rat liver cytochrome P450-linked enzymes, namely, aniline hydroxylase and aminopyrine demethylase. Only hexane and chloroform extracts were able to inhibit the activities of these two enzymes. Both hexane and chloroform extracts inhibited the activity of aniline hydroxylase to a much greater extent than that of aminopyrine demethylase. The

amounts of hexane extract capable of inhibiting 50% of aniline hydroxylase and aminopyrine demethylase activities were approximately 0.16 mg and 0.31 mg, respectively, while those of chloroform extract were approximately 0.09 mg and 0.28 mg, respectively. And again, methanol extract was unable to inhibit the activities of these two enzymes, although it was tested up to 1 mg.

### Discussion

Results of the present study demonstrate that Chinese radish does not contain any compound mutagenic toward *S. typhimurium*, either strains TA98 or TA100 whether tested with or without metabolic activation. Brussels sprouts have also been shown not to be mutagenic to these tester strains (11). However, we have recently found that collards, celery cabbage, and cabbage, which are also the cruciferous vegetables do contain some weak mutagens toward strain TA98 when tested in the absence of metabolic activation (15). Many other vegetables such as lettuce, rhubarb, string beans, paprika, and braken fern have also been shown to have mutagenic activities. Quercetin may be partly responsible for the mutagenic activity found in lettuce and string beans, and emodin has been shown to be the single contributor to the mutagenic activity observed in rhubarb (11).

Interestingly, our results in the antimutagenicity study reveal clearly that Chinese radish contains some anti-mutagens capable of inhibiting both direct mutagens such as AF-2 and  $\text{NaN}_3$  and indirect mutagens such as  $\text{AFB}_1$ .

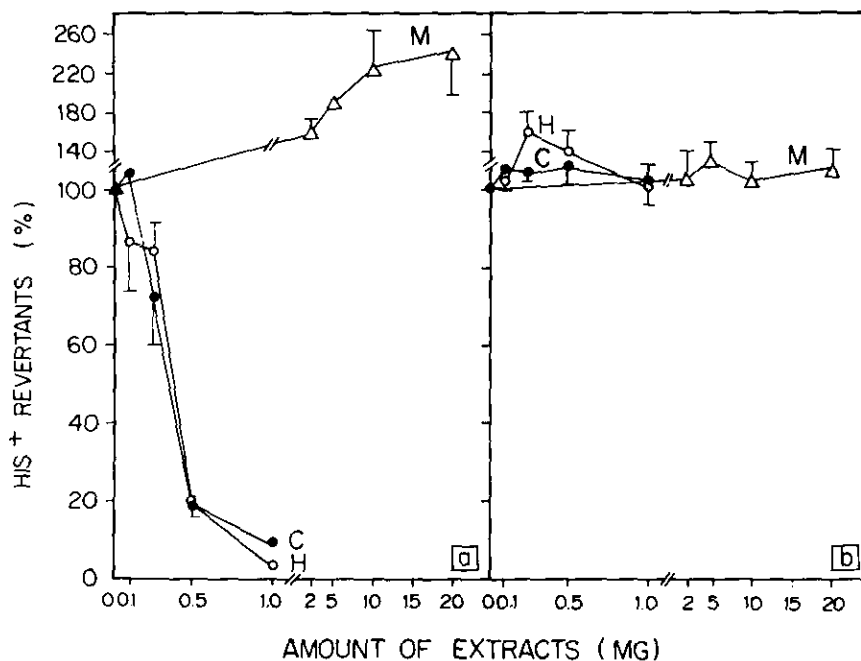


FIGURE 2. Effects of Chinese radish extracts on the mutagenicities of aflatoxin  $\text{B}_1$  ( $\text{AFB}_1$ ) (a) and benzo[a]pyrene (BaP) (b) toward *S. typhimurium* TA100 in the presence of S-9 mix. S-9 mix used for  $\text{AFB}_1$  and BaP mutagenesis assay contained 200 and 100  $\mu\text{L}/\text{mL}$ , respectively, of S-9 fraction. The numbers of His<sup>+</sup> revertants induced by  $\text{AFB}_1$  (0.03  $\mu\text{g}$ ) and BaP (5  $\mu\text{g}$ ) in the absence of any extract were  $1,291 \pm 100$  and  $1,219 \pm 133$ , respectively. For other details, see Figure 1.

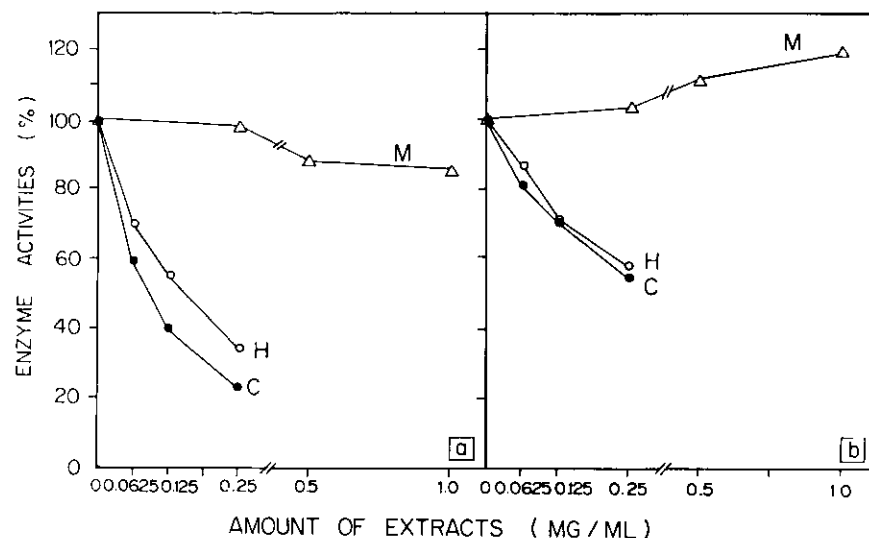


FIGURE 3. Effects of Chinese radish extracts on the activities of rat liver aniline hydroxylase (a) and aminopyrine demethylase (b). The enzyme activities in the presence of hexane (○), chloroform (●), and methanol (△) extracts are shown as the percentage of those assayed without any extract. The activities of aniline hydroxylase and aminopyrine demethylase in the absence of vegetable extracts were 0.313 nmole *p*-aminophenol formed/min/mg protein and 256.7 nmole formaldehyde formed/min/mg protein, respectively. Results are means of two separate experiments.

These compounds are nonpolar in nature because they were extractable only by nonpolar solvents such as hexane and chloroform. However, these two extracts were unable to inhibit the mutagenicity of BaP, which is also an indirect mutagen. For other vegetables, we have recently found that collard, cabbage, celery cabbage, and bitter melon also contain nonpolar antimutagens capable of inhibiting the mutagenicity of AFB<sub>1</sub>, but not or only slightly inhibiting that of BaP. These vegetable extracts could not inhibit the mutagenicity of direct mutagen such as AF-2 (15). Wall et al. (10) have also reported that chloroform extracts of cabbage, collards, broccoli, and turnip greens were able to inhibit the mutagenicity of acetylaminofluorene but not that of BaP.

The mechanism by which the hexane and chloroform extracts of Chinese radish inhibited the mutagenicities of AF-2, NaN<sub>3</sub>, and AFB<sub>1</sub> is not known. However, because these fractions inhibited both direct and indirect mutagens, it may be suggested that the antimutagens mainly inhibit some steps in the mutation process rather than generally interacting with some enzymes necessary for activation of chemical mutagens.

However, we have also found that hexane and chloroform extracts of Chinese radish, but not the methanol extract, were able to inhibit the activities of two rat liver cytochrome P450-linked enzymes, namely, aniline hydroxylase and aminopyrine demethylase. The activity of the aniline hydroxylase was inhibited to a greater extent than aminopyrine demethylase. Thus, the inhibition on AFB<sub>1</sub> mutagenicity of these two extracts may also be partly due to the interaction with enzyme(s) in the liver homogenate that are necessary for activation of this mutagen. And together with the finding that different cytochromes P450 are involved in the metabolic activation of AFB<sub>1</sub> and BaP (16), the lack of inhibitory effect on BaP mutagenicity may

thus be partly due to the inability of the hexane and chloroform extracts to inhibit the enzyme(s) necessary for metabolic activation of this mutagen. Lettuce and string bean extracts as well as some natural compounds have been reported to inhibit the mutagenicities of BaP and cigarette smoke condensate, and the mechanism was suggested to be the interaction between the antimutagen and enzyme(s) in the liver homogenate (11).

From all results in the present study, it may be concluded that Chinese radish does not contain any mutagen toward *S. typhimurium* but contains some antimutagens to both direct and some indirect mutagens. Thus, we suggest that people increase their consumption of this vegetable. The capability of this vegetable to induce the mixed-function oxidase system and the activity of glutathione-S-transferase as well as the inhibitory effect on the AFB<sub>1</sub>-induced liver carcinogenesis in rats is being investigated in our laboratory.

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